COMPARISONS OF SOIL ORGANIC MATTER AND ITS FRACTIONS BY PYROLYSIS MASS-SPECTROMETRY

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ABSTRACT

Pyrolysis mass-spectra from a sample of the A_1-horizon of a soil from southern Spain showed predominant peaks related to furan derivatives similar to those observed from complex polysaccharides in which not only hexoses but also pentoses and deoxyhexoses were constituent units. Smaller peaks, typical for protein materials and phenolic units, were also observed. On the other hand, typical peaks for the methoxyphenols of lignins were very small and indicated only limited amounts of undecomposed lignin residues in this soil sample. Peaks related to benzene or toluene were also very small.

Humic acid samples from this soil showed much more prominent signals related to protein materials, benzene and phenolic derivatives and weaker polysaccharide-related signals than did the entire sample. Typical lignin related peaks were small or insignificant. Spectra from the grey or brown humidic acid fractions were much like those of the parent humic acid. Brown humic acid, however, showed stronger signals for nitrogen and sulphur compounds, indicating a higher content of protein-like materials in this fraction. Preparations of humic acid hydrolyzed by 6 *N* HCl showed in their pyrolysis products a marked increase in phenols and methoxyphenols.

In its pyrogram, humin resembled humic acid, but signals for complex polysaccharides were more evident. Lignin-like materials seem not to be higher in this fraction. Hymatomelanic acid showed prominent signals related to polysaccharides and lignin. Pyrograms from the soil polysaccharides showed the characteristic pattern of a complex polysaccharide with the presence of fragments from polymers of amino acids or amino sugars. Fulvic acid spectra showed obvious dissimilarities to those from humic acid in that signals for protein, as well as those related to phenols, were low. Depending upon the isolation method, the fulvic acid preparations showed differing signals related to polysaccharide or phenolic materials.

INTRODUCTION

Curie point pyrolysis in direct combination with low voltage mass spectrometry has been used for the identification of bacteria and biopolymers

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(Meuzelaar et al., 1973, 1977b; Maugh, 1976). Nagar et al. (1975) further indicated that the technique was promising for soil humus research. A more extensive study has been made by Meuzelaar et al. (1977a) by comparing humic acids from soils, peats and composted straw, as well as fungal humic acid-type melanins and lignins. Humic acids from different soils and peats and most of the fungal melanins gave similar pyrograms with typical ion series probably related to protein-like materials, polysaccharides and phenolic and aromatic compounds. These ion series mainly showed variations with respect to peak height. Furthermore, humic acids showed more or less significant ion series demonstrated as typical for lignin materials (Meuzelaar et al., 1977a; Haider et al., 1977).

In the experiments described in this paper, pyrolysis mass spectra were prepared from untreated soil samples and for several fractions of the organic matter extracted from that sample. Principal fractions were humic and fulvic acids, humin and polysaccharides. In addition, some subfractions, such as B and D fractions from fulvic acid, grey and brown humic acids and hymatomelanic acid, were analyzed. Last of all, spectra were prepared for the soil sample residue after extractions, for the soil sample hydrolyzed with HCl, and for the HCl-hydrolyzed humic acid.

MATERIALS AND METHODS

The soil sample used in this study was from the A_1 horizon of a brown soil on granite (Typic Xerochrept) in the northern part of the province of Huelva, southwestern Spain. The altitude was 480 m and the vegetation consisted of an uncultivated prairie with gramineous plants, *Medicago* and *Trifolium*. The sample was taken to a depth of 0—10 cm and had a pH of 5.6 in H_2O, a carbon content of 3.5% and a nitrogen content of 0.4%.

The air-dried sample was powdered to pass a 2-mm sieve. Before extraction plant debris was removed by flotation. Batches of 400 g of the sieved sample were extracted with cold 1 : 1 solution of 0.1 *M* $Na_4P_2O_7$ and NaOH under N_2, as described by Vila et al. (1974). The extraction was repeated with fresh solvent until no appreciable amounts of humic acid (HA) could be removed (about 9 treatments). The extracts were centrifuged at 10,000 g, acidified to pH 2 with HCl and the precipitated humic acid was removed by centrifugation. It was dissolved again in 0.1 *N* NaOH, centrifuged at 15,000 g to remove mineral residues, then acidified and washed with 0.1 *N* HCl until colorless. After a deashing treatment with a dilute HF/HCl-solution (5 ml 48% HF + 5 ml 36% HCl + 990 ml H_2O) for 24 h and subsequent centrifugation it was repeatedly washed with 0.1 *N* HCl dialyzed against distilled water for one week and freeze-dried.

From the supernatant solution after the HA precipitation, the fulvic acid (FA) was isolated by Polyclar AT (polyvinyl-pyrrolidone from Serva, Heidelberg). The Polyclar was pretreated by washing several times with 0.1 *N* HCl and water, then the fulvic acid was adsorbed on this Polyclar by filtration

and recovered afterwards by elution with 0.1 *N* NaOH (Goh, 1970). This eluate was filtered and adjusted to pH 4 and then the FA was precipitated by adding $FeCl_3$. This precipitate was washed several times to remove chloride and then treated with Amberlite IR 120 in the H^+ form by shaking to solubilize the FA at a pH of about 2.5 to 3. Alternatively, the FA was isolated according to Forsyth (1947) by adsorption on charcoal. Only fraction B, eluted with acetone-water, and fraction D, eluted with 0.1 *N* NaOH, were retained. Fraction D was further purified as indicated for the fulvic acid eluted from Polyclar and fraction B was directly lyophilized. To isolate humin, the soil sample after extraction with pyrophosphate-NaOH, was repeatedly washed with distilled water and treated afterwards several times with the HF/HCl solution for 24 h. Then the sample was washed, until the water reached a pH of 6, and extracted two times with 0.5 *N* NaOH. The alkaline extract was treated as described earlier for the HA but without the de-ashing procedure.

To isolate the polysaccharide fraction, the slightly yellow Polyclar filtrate was freeze-dried, dissolved in a small amount of water and dialyzed for one week against distilled water and then lyophilized.

Grey and brown HA's were obtained according to Flaig et al. (1955). Each subfraction was dialyzed and freeze-dried. Hymatomelanic acid was obtained by shaking the freeze-dried HA several times with ethanol until the solution was colorless. Then the ethanol was evaporated at 35°C and the residue was suspended in distilled water and freeze-dried.

Analysis of the C, H, N and ash values was made by Mikroanalytisches Laboratorium A. Bernhardt, Elbach (Germany), oxygen was calculated by difference to 100%. Hydrolysis of the soil sample and the humic acid was accomplished with 6 *N* HCl in a sealed tube under N_2 for 24 h at 105°C. The hydrolyzed residues were washed and lyophilized.

The pyrolysis mass spectrometry method has been described in detail by Meuzelaar et al. (1973). The special procedures to pyrolyse humic compounds were described by Meuzelaar et al. (1977a) and Haider et al. (1977). The sample was dispersed in methanol, coated on a ferromagnetic wire and pyrolyzed at 510°C. In contrast to earlier methods (Nagar et al., 1975; Meuzelaar et al., 1977a) in which NaOH was used as a dispersing agent, methanol was used in these studies because alkali causes salt formation and complicates drying of the sample coated on the wire.

RESULTS

Table I shows the elementary composition of the isolated soil organic matter fractions on a dry and ash-free basis and the yields of the respective fractions in grams obtained from 100 g of soil sample.

Fig. 1. presents pyrolysis-mass spectra for the total soil sample, the humic acid and the humin and for the soil sample after extraction. The spectrum for fulvic acid, also one of the more prominent fractions, is shown along

TABLE I

Analytical values of the soil organic matter fraction

	Yield*	C (%)	H (%)	N (%)	O (%)	Ash (%)
Humic acid	3.0	51.4	5.8	4.1	38.7	5.5
Humin	0.2	55.1	6.6	4.2	34.1	2.2
Polysaccharide	0.4	37.8	6.8	2.1	53.3	22.0
Fulvic acid (Polyclar)	0.3	47.9	5.2	2.6	44.3	11.5
Fulvic acid (fraction D)	0.2	39.5	4.3	2.5	53.7	21.1
Fulvic acid (fraction B)	0.1	49.9	6.3	1.4	42.4	6.5
Grey humic acid	1.0	49.4	6.9	3.9	39.8	12.2
Brown humic acid	1.8	50.6	5.9	4.7	38.8	4.5
Hymatomelanic acid	0.1	57.8	7.5	1.1	33.6	2.5

* Respective fractions in grams as obtained from 100 g of soil sample.

with that for the polysaccharide fraction in Fig. 2. The most prominent peaks in the pyrolysis spectrum of the whole soil sample agree with those obtained from a complex polysaccharide. Peaks at m/e 43, 60, 68, 72, 82, 84, 96, 98, 102, 110, 112, 126 were also obtained by pyrolysis of cellulose (Haider et al., 1977). They were shown to be related to acetic acid (m/e 60), furan (m/e 68), methylfuran (m/e 82), hydroxyfuran (m/e 84), furfural and/or pyrone (m/e 96), and furfuryl alcohol (m/e 98) (Posthumus et al., 1974). However, other peaks point to a more complex polysaccharide pattern, especially those at m/e 114 and 128. Smaller peaks at m/e 67, 81, 95 and 117, are typical for protein materials and are likely to represent (alkyl) pyrroles and indole. A prominent peak at m/e 17 shows the presence of ammonia. Peaks at m/e 124, 138, 150, 152 and 164, strongly suggest the presence of small amounts of guaiacyl units derived from lignin residues (Meuzelaar et al., 1977a; Haider et al., 1977). Peaks at m/e 78 and 92 are small and show that only very limited amounts of benzene and toluene were released through pyrolysis.

Humic acid in comparison to the total soil sample shows much more prominent signals related to ammonia (m/e 17) and to the sulfide (m/e 34, 48), pyrrole (m/e 67, 81, 95), indole (m/e 117, 131), benzene (m/e 78, 92, 106) and phenol (m/e 94, 108, 120, 124, 138, 150) series. Peaks related to polysaccharides are relatively small in the spectrum for humic acid as compared to that for the whole sample. Signals indicative of ligninlike material (m/e 124, 138, 150, 154, 162 and 164) are small compared to those obtained by pyrolysis of isolated lignin.

The pyrogram for humin resembles that of humic acid. The ion intensities of the peaks from protein-like and aromatic compounds are similar; however, signals from polysaccharides, including peaks at m/e 112, 114, 126 and 128, are more evident. Signals at m/e 124, 138 and 150 are comparable and indicate that lignin residues are not specifically enriched in humin.

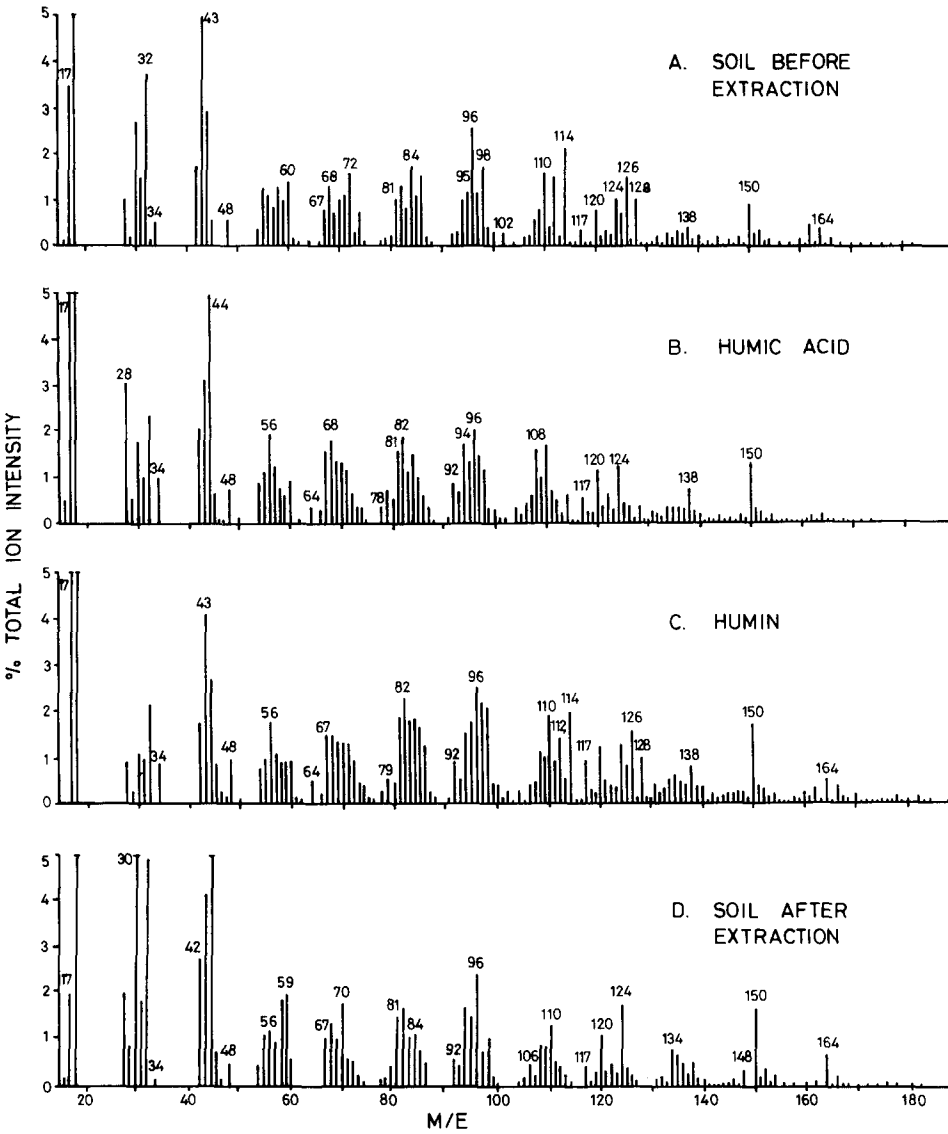

Fig. 1. Pyrolysis mass spectra of the whole soil sample and some of its organic fractions as well as the soil residue after extraction.

The pyrolysis mass spectrum of the soil sample after extraction still indicates the presence of organic materials related to those of the unextracted soil sample. Protein-related peaks are not essentially decreased and only the ammonia peak at m/e 17 is lower. Polysaccharide peaks, especially those at m/e 102, 112, 114, 126 and 128, show a decrease and therefore indicate the disappearance of the more complex polysaccharide material. Phenol and lignin-derived peaks increase somewhat, meaning that some non-extractable

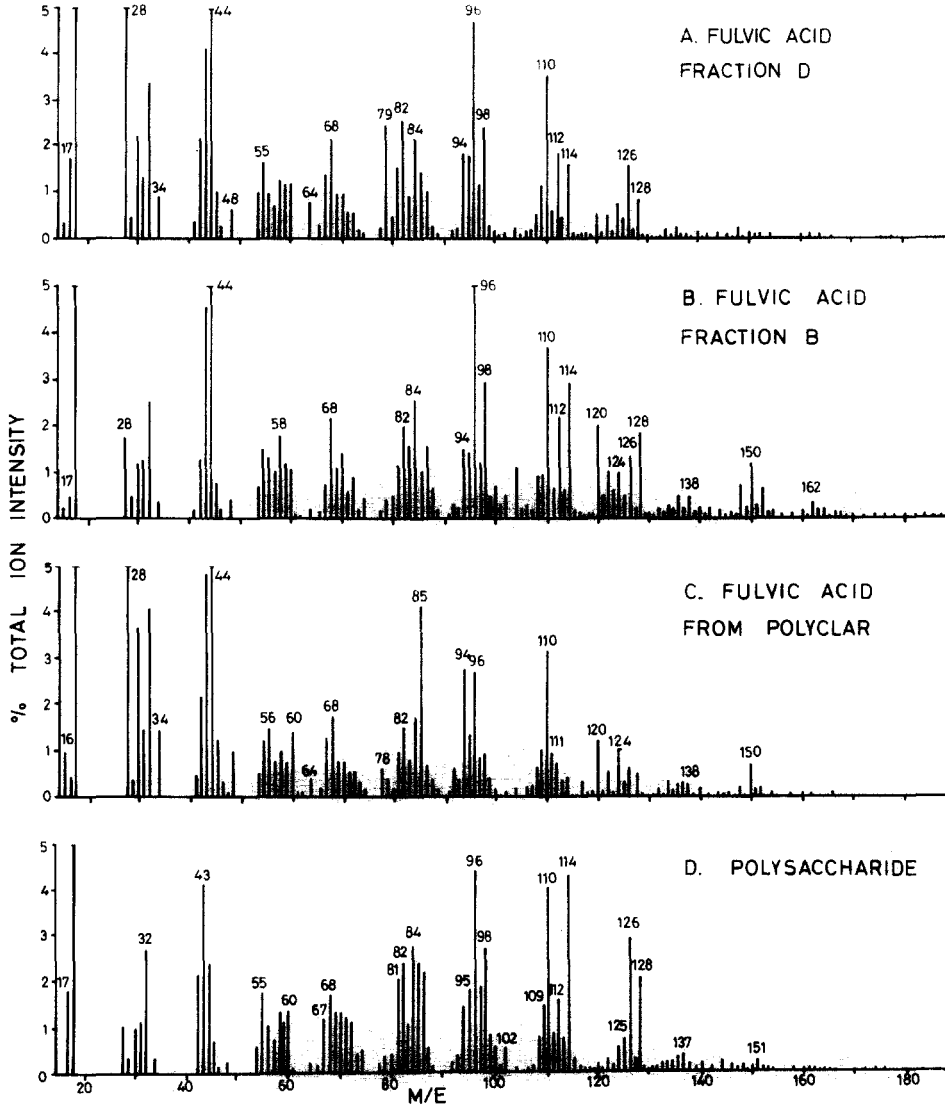

Fig. 2. Pyrolysis mass spectra of different fulvic acid preparations purified by Polyclar or charcoal treatments and a polysaccharide fraction isolated from Polyclar filtrate.

lignified plant material may have remained, whereas the more complex polysaccharide material was extracted by the alkali.

Fig. 2 shows the spectra of several fulvic acid fractions isolated by different methods. The spectra provide evidence that the method of preparation strongly influences the composition of the sample. Both preparations isolated from the charcoal (fraction D and B) show dominant signals similar to those observed in pyrograms from complex polysaccharides (m/e 68, 82, 84, 96, 98,

110, 114, 126 and 128). Furthermore, nitrogen-containing fragments are low in both preparations. Fraction B differs from D by more prominent signals of phenolic material, especially those in higher mass range (m/e 124, 138, 150, 152, 162, 164).

The preparation isolated with Polyclar resembles humic acid more than those isolated from charcoal by different eluants. It shows higher phenol related peaks at m/e 94, 120, 124, 138 and 150 than fraction D which was obtained from charcoal by elution with NaOH. The high affinity of Polyclar for phenols, as indicated by its use to remove polyphenols from wines and beers, may explain the higher phenol content in this fulvic acid preparation. In addition, this preparation contains residues of polyvinylpyrrolidone, as evidenced by the peaks at m/e 85 and 111 (pyrrolidone and vinylpyrrolidone).

The polysaccharide fraction (Fig. 2D) shows upon pyrolysis signals at m/e 43, 60, 68, 82, 84, 96, 110, 112, 114, 126 and 128, which are indicative of a complex polysaccharide. The intensity of these signals is more pronounced than in fulvic acid preparations B and D. However, signals from phenolic and aromatic compounds are weak as compared to those of fulvic acid fraction isolated from charcoal; especially so in fraction B. Possible fragments of amino sugar origin appear to be relatively low, as evidenced by signals at m/e 95, 109, 125, 137 and 151 (Meuzelaar et al., 1974).

Fig. 3 shows the spectra of grey and brown humic acids, hymatomelanic acid and the hydrolyzed parent humic acid. Both, grey and brown humic acid are much like the parent humic acid. However, grey humic acid shows a much lower ammonia peak and a decrease in some of the peaks attributable to proteins. Polysaccharide peaks, however, are similar or even more evident than for the parent preparation (m/e 68, 62, 84, 96,,112, 114, 126 and 128). Brown humic acid is much richer in protein-related materials, indicated by m/e 34, 48 (sulfides), 67, 81, 96 (pyrroles), 117, 131 (indoles) and m/e 78, 92, 106 (benzenes). Also peaks indicative of (alkyl) pyridines (m/e 79, 93, 107) are higher but polysaccharide-related peaks are lower. More prominent also are phenol and methoxyphenol peaks.

Hymatomelanic acid exhibits prominent peaks for methanol and ethanol (m/e 32 and 46). Methanol was used as a solvent to apply the sample to the wire and ethanol as an extractant to isolate hymatomelanic acid from the humic acid. The peaks show that both solvents are difficult to remove. Predominant in this spectrum are (alkyl) furan and (methoxy) phenol peaks (m/e 68, 82, 96, 110; 94, 108, 120, 122, 124, 134, 138, 150, 152). This gives the impression that hymatomelanic acid consists of materials rich in polysaccharides and lignins removable from humic acid by extraction with ethanol.

The last spectrum in Fig. 3 represents a pyrogram of the 6 *N* HCl hydrolyzed humic acid. Compared with the nonhydrolyzed preparation in Fig. 1, polysaccharide and protein related peaks have decreased greatly whereas signals related to phenols and/or methoxyphenols (m/e 94, 108, 110 (?), 120, 122, 124 and 134) have increased. In comparison with the nonhydro-

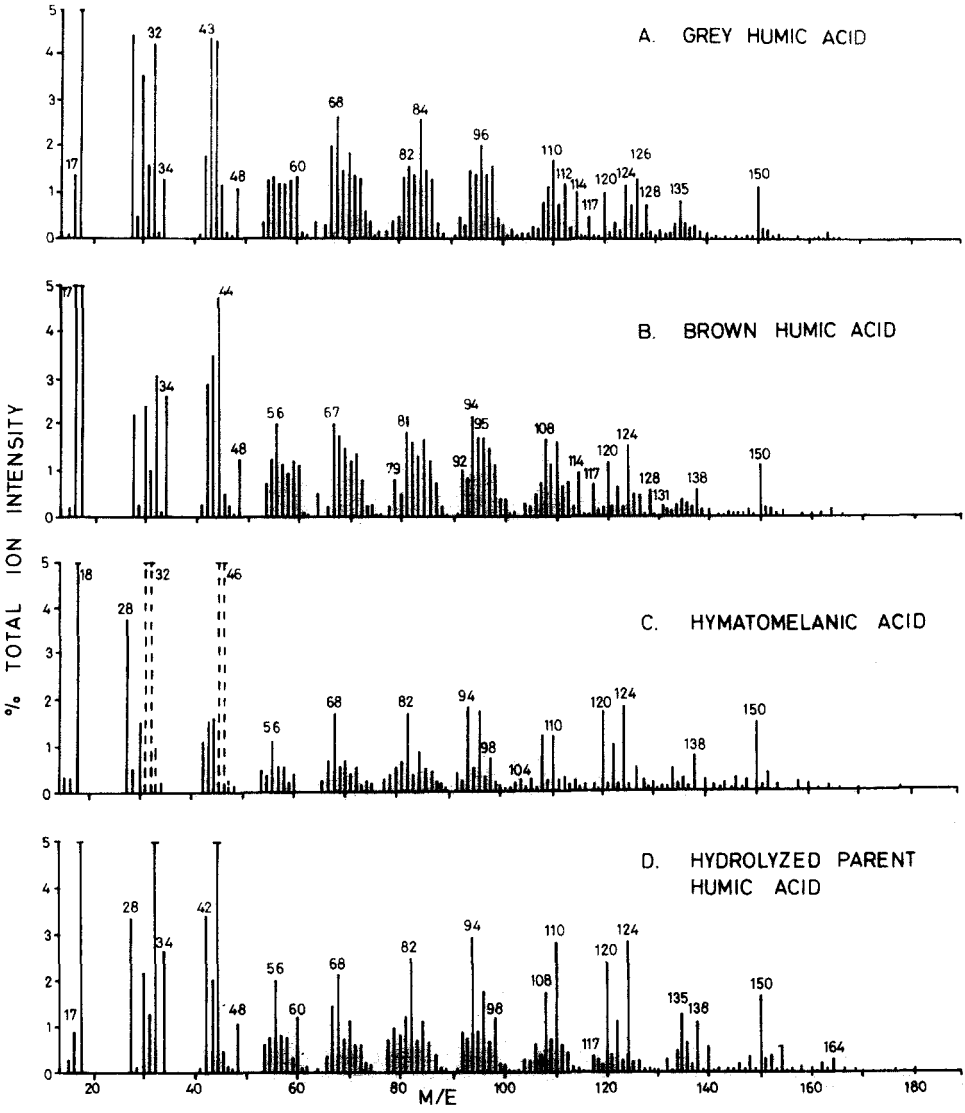

Fig. 3. Pyrolysis mass spectra of subfractions from humic acid and the 6*N* HCl hydrolyzed parent humic acid.

lyzed parent material where polysaccharide and protein-related peaks are the most prominent signals, the hydrolyzed material shows mainly phenolic peaks suggesting a phenolic framework with the presence of some lignin-like residues. Even after hydrolysis some peaks apparently related to nitrogen and polysaccharide compounds are still present, though markedly decreased.

The pyrogram of the 6 *N* HCl hydrolyzed soil sample (not given) showed in comparison with the untreated sample a decrease of ammonia (m/e 17)

and of the characteristic peaks related to proteins or polysaccharides. The pentose and deoxyhexose peaks at m/e 114 and128 had disappeared. The phenolic signals at m/e 94, 108, 120, 122 and 124 were greatly increased.

Hydrolyzed melanins from several soil fungi (Meuzelaar et al., 1977a) like hydrolyzed humic acids showed prominent phenolic series at m/e 94, 108, 110, 120, 122, 124 and 160. However, typical peaks related to lignin materials at m/e 138, 150, 152 and 164 were missing or much less prominent in the pyrograms of the hydrolyzed fungal materials. The peak at m/e 124 was present for both materials, it could be related either to guaiacol or to one or more dihydroxytoluenes. These latter compounds were shown to be building blocks of fungal melanins (Martin and Haider, 1971).

DISCUSSION

The pyrogram of the total soil sample prior to extraction of organic matter showed a predominance of peaks related to complex polysaccharides. Peaks from aromatic, phenolic or protein-containing materials were also evident but not as prominent as those from polysaccharides. This predominance of polysaccharides does not seem to be a peculiarity of this specific soil but was similarly observed in the pyrograms for samples of a podzol and various other soils (Haider et al., 1977, and unpublished data). Pyrolysis gas chromatography studies of the A horizons of several soil types (Bracewell and Robertson, 1976, 1977) also showed 2-furaldehyde and 5-methyl-2-furaldehyde to be the most prominent components. With increasing depth, however, the relative amounts of these furaldehydes decreased. The total polysaccharide content of a soil can only be roughly estimated and the polysaccharide fraction obtained by mild extraction represents only a small percentage of the total content. Even if this total content is determined by colorimetric measurements after hydrolysis it seems to be arbitrary (Gupta, 1967). Data for amounts in soil therefore, differ greatly, ranging from 5 to 25%, but might be even higher (Martin, 1971). Polysaccharides probably constitute by quantity one of the most important fractions in soil organic matter. They are formed from complex sugar units of hexoses and pentoses, including some deoxyhexoses, tetroses, trioses, amino sugars and uronic acid as well (Martin, 1971; Guckert, 1973; Cheshire et al., 1974). The qualitative composition of these units seems not to differ much in different soil types (Forsyth, 1950; Cheshire et al., 1974). This complexity of the sugar units in the soil polysaccharides is also obvious in the pyrograms from the untreated soil sample and from several of the fractions as indicated by peaks not only related to hexose units but also to deoxyhexose and pentose and possibly (N-acetyl) aminohexose units. Signals at m/e 114 and 128 have been shown to be related to pentose units and deoxyhexose units, respectively (Posthumus et al., 1974; Weijman, 1977). They were not present in the pyrolysis spectrum of cellulose. These complex polysaccharides are easy to extract; after extraction the soil sample did not show the peaks at m/e 112, 114, 126 and 128.

The second unique fraction of soil organic matter appears to be the humic acid. Compared to the pyrogram of the total soil sample, peak intensities of aromatic, phenolic and protein-related peaks have increased. However, the main peaks in the spectrum of the humic acid fraction are already present in the pyrogram for the total soil sample. This indicates that humic acids are a real part of the organic matter and are not formed as artifacts during extraction. Pyrograms from humic acid samples of different soils showed complex but highly similar spectra (Nagar et al., 1975; Meuzelaar et al., 1977a). This similarity is indicated especially by the presence of homologous ion series of "sulfides" (m/e 34, 48 and 62), "pyrroles" (m/e 67, 81 and 95), "benzenes" (m/e 78, 92 and 106), "phenols" (m/e 94, 108 and 122) and "indoles" (m/e 117, 131 and 145) with variable intensities (Meuzelaar et al., 1977a). In addition to these series, peaks from polysaccharides and lignins also occur with variable intensities. The latter peaks are sometimes quite obvious, e.g. in a humic acid sample from an Indian red soil (Nagar et al., 1975) or from a chernozem soil. In other humic acids, however, lignin-related peaks are sometimes very small or not detectable, e.g. in humic acids from podzol, investigated by Meuzelaar et al. (1977a) and Haider et al. (1977). Bracewell and Robertson (1976) observed by gas chromatography of pyrolysates from a podzol that guaiacol and 4-methyl-guaiacol occurred only in the surface horizons and not in the deeper layers. They concluded from this finding that lignin material is rapidly degraded in the deeper layers. There is great similarity between pyrograms of grey and brown humic acids and humic acid itself, which supports the idea of basic similarities in chemical composition. A higher protein content of the brown humic acid is indicated by higher peaks of nitrogen and sulfur containing compounds.

Another fraction similar to humic acid but richer in polysaccharide material is humin. According to Guckert (1973) and also to Acton et al. (1963) humins generally contain high amounts of polysaccharides. This high polysaccharide content is shown by the pyrogram of humin which also indicates the presence of more complex polysaccharides than those in humic acid.

The fulvic-acid fraction from the soil sample has obvious relationships to polysaccharide-like materials. However, the method of isolation determines whether the pyrogram shows more or less prominent polysaccharide peaks. In this respect the Polyclar preparation shows peaks from polysaccharides besides those from phenol and aromatic materials. These latter peaks are not so obvious in the "D" fraction of fulvic acid, isolated according to Forsyth (1947) by extraction from the preparation adsorbed on charcoal. It was observed by several authors (e.g. Swincer et al., 1968; Martin, 1971) that polysaccharides can be separated from fulvic acid. However, this separation was never complete. Ogner and Schnitzer (1971), and Schnitzer and Khan (1972) indicate that phenolic and benzenecarboxylic acids, linked by hydrogen bridges, are major constituents of soil fulvic acids. However, Anderson and Russel (1976) found in a fulvic acid preparation that aromatic molecules are of minor importance. They furthermore concluded from IR

spectra that this fulvic acid preparation was possibly related to polymaleic acid. Rosochacka (1969) suggested from IR studies of fraction B of fulvic acid the presence of sugars in this fraction but did not detect typical bands of aromatic compounds. The similarity between the pyrogram from the fulvic acid fraction separated from Polyclar and that from humic acid indicates a correspondence in chemical structure after removal of the abundant polysaccharide materials from fulvic acid.

The appreciable contribution of polysaccharide materials to soil organic matter indicated by the pyrograms is somewhat astonishing in view of findings by several authors (see also Martin et al., 1974) of a rapid degradation of plant polysaccharides in soil. Together with the complex pattern of these soil polysaccharides, indicated by the pyrograms and by observations of Swincer et al. (1968), their presence lends credence to the idea of microbial biosynthesis of soil polysaccharides discussed by Martin (1971) and Cheshire et al. (1974). Lignins, on the other hand, which are reported to be more stable against biodegradation than polysaccharides (Umbreit, 1962), seem to be readily altered or decomposed in soil as indicated by the relatively weak typical lignin signals in pyrograms of whole soil samples and the several organic matter fractions. This agrees with observations of low methoxyl contents in soil organic matter and small yields of typical lignin fragments upon nitrobenzene or permanganate oxidation (Bremner, 1955; Morrison, 1963; Wildung et al., 1970; Schnitzer and Khan, 1972) of soil organic matter fractions.

Pyrolysis mass spectrometry as a "survey" technique gives preliminary informations about the compisition of soil organic matter in a relatively short time and without extensive chemical treatments. However, it has limitations in explaining biochemical transformations of plant materials into humus because it cannot discriminate between ions of the same mass to charge ratio but with different chemical constitutions that may contribute to a single mass peak.

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